Listing of Claims

1. (currently amended) A nucleic acid vector comprising:

first and second nucleotide sequences corresponding to nucleotide sequences flanking a predetermined insertion site in the RL1 locus of the genome of a selected herpes simplex virus (HSV); and

(I) a cassette located between said first and second nucleotide sequences comprising nucleic acid encoding:

(a) one or a plurality of insertion sites; and

(b) a ribosome binding site or a regulatory nucleotide sequence; and

(c) a marker,

wherein the nucleic acid encoding the one or plurality of insertion sites is/are arranged upstream (5′) of the ribosome binding site or the regulatory nucleotide sequence and the nucleic acid encoding the ribosome binding site or the regulatory nucleotide sequence is arranged upstream (5′) of the marker; or

(II) a nucleic acid cassette located between said first and second nucleotide sequences comprising:

(a) a third nucleotide sequence being of interest;

and nucleic acid encoding:

(b) a ribosome binding site or a regulatory nucleotide sequence; and

(c) a marker,

wherein the nucleotide sequence of interest is arranged upstream (5′) of the ribosome binding site or the regulatory nucleotide sequence and the ribosome binding site or the regulatory nucleotide sequence is arranged upstream (5′) of the marker.

2. (cancelled)

3. (currently amended) ~~A~~ The vector ~~as claimed in~~ of claim 1 ~~or claim 2~~ wherein the ribosome binding site comprises an internal ribosome entry site (IRES).

4. – 5. (cancelled)

6. (currently amended) ~~A~~ The vector ~~as claimed in claim 4 or~~ of claim ~~5~~ 1 wherein said regulatory nucleotide sequence is operably linked to said marker.

7. (currently amended) ~~A~~ The vector ~~as claimed in any one~~ of ~~claims 4 to 6~~ claim 1 wherein said regulatory nucleotide sequence comprises a constitutive or inducible promoter.

8. (currently amended) ~~A~~ The vector ~~as claimed in~~ of claim ~~2~~ 1 ~~or claim 5~~ wherein the nucleotide sequence of interest encodes an heterologous polypeptide.

9. (currently amended) ~~A~~ The vector as claimed in claim 8 wherein the heterologous polypeptide is selected from the group consisting of: a bacterial polypeptide; a mammalian polypeptide; a human polypeptide.

10. (currently amended) ~~A~~ The vector as claimed in claim 8 wherein the heterologous polypeptide is selected from the group consisting of: Sodium iodide symporter (NIS); Nitroreductase (NTR); *E. coli* NTR; Endothelial nitric oxide synthase (eNOS); Granulocyte Macrophage Colony-Stimulating Factor (GM-CSF); a cytokine.

11. (currently amended) ~~A~~ The vector ~~as claimed in~~ of claim ~~2~~ 1 ~~or claim 5~~ wherein the nucleotide sequence of interest encodes a selected antisense nucleic acid or siRNA.

12. (currently amended) ~~A~~ The vector ~~as claimed in any one~~ of ~~claims 2, 3, 5, 8 to 11~~ claim 1 wherein the cassette further comprises a regulatory nucleotide sequence located upstream (5′) of the nucleotide sequence of interest which has a role in regulating transcription of the nucleotide sequence of interest.

13. (currently amended) ~~A~~ The vector ~~as claimed in any one~~ of ~~claims~~ claim 1~~, 3, 4, 6 or 7~~ wherein the cassette further comprises a regulatory nucleotide sequence located upstream (5′) of the insertion site(s).

14. (currently amended) ~~A~~ The vector ~~as claimed in any one~~ of ~~claims~~claim 1~~, 3, 4, 6, 7, or 13~~ wherein the cassette comprises a plurality of said insertion sites.

15. (currently amended) ~~A~~ The vector ~~as claimed in any one~~ of ~~claims~~claim 1~~, 3, 4, 6, 7, 13 or 14~~ wherein each insertion site is formed by nucleic acid encoding a restriction endonuclease site.

16. (currently amended) ~~A~~ The vector ~~as claimed in any one~~ of ~~claims~~claim 1~~, 3, 4, 6, 7, 13 , 14 or 15~~ wherein the insertion sites comprise one or more of the ClaI, BglII, NruI and XhoI restriction endonuclease sites.

17. (currently amended) ~~A~~ The vector ~~as claimed in any one~~ of ~~claims~~claim 1 ~~to 16~~ wherein the first and second nucleotide sequences each comprise sequence corresponding to nucleotide sequences in the RL terminal or internal repeat region of the genome of the selected HSV.

18. (currently amended) ~~A~~ The vector ~~as claimed in any one~~ of ~~claims~~claim 1 ~~to 17~~ wherein said first and second nucleotide sequences correspond to nucleotide sequences flanking a predetermined insertion site formed in, or comprising all or a part of, the ICP34.5 protein coding sequence of the genome of a selected herpes simplex virus.

19. (currently amended) ~~A~~ The vector ~~as claimed in any one~~ of ~~claims~~claim 1 ~~to 18~~ wherein said first and second nucleotide sequences comprise contiguous portions of nucleotide sequence of the ICP34.5 gene of a herpes simplex virus.

20. (currently amended) ~~A~~ The vector ~~as claimed in any one~~ of ~~claims~~claim 1 ~~to 19~~ wherein said first and second nucleotide sequences comprise contiguous portions of nucleotide sequence encoding the ICP34.5 gene product of a herpes simplex virus.

21. (currently amended) ~~A~~ The vector ~~as claimed in any one~~ of claim 1 ~~to 20~~ wherein the first and second nucleotide sequences have at least 60% sequence identity to their corresponding sequence in the viral genome.

22. (currently amended) ~~A~~ The vector ~~as claimed in any one~~ of ~~claims~~claim 1 ~~to 20~~ wherein said first and second nucleotide sequences hybridise to their corresponding nucleotide sequence in the HSV genome, or its complement, under high or very high stringency conditions.

23. (currently amended) ~~A~~ The vector ~~as claimed in any one~~ of ~~claims~~claim 1 ~~to 22~~ wherein the marker is a defined nucleotide sequence encoding a polypeptide.

24. (currently amended) ~~A~~ The vector ~~as claimed in any one~~ of ~~claims~~claim 1 ~~to 23~~ wherein the marker comprises the Green Fluorescent Protein (GFP) protein coding sequence or the enhanced Green Fluorescent Protein (EGFP) protein coding sequence.

25. (currently amended) ~~A~~ The vector ~~as claimed in any one~~ of ~~claims~~claim 1 ~~to 22~~ wherein the marker comprises a defined nucleotide sequence detectable by hybridisation under high stringency conditions with a corresponding labelled nucleic acid probe.

26. (currently amended) ~~A~~ The vector ~~as claimed in any one~~ of ~~claims~~claim 1 ~~to 25~~ wherein the cassette further comprises nucleic acid encoding a polyadenylation sequence located downstream (3′) of the nucleic acid encoding the marker.

27. (currently amended) ~~A~~ The vector as claimed in claim 26 wherein the polyadenylation sequence comprises the Simian Virus 40 (SV40) polyadenylation sequence.

28. (currently amended) ~~A~~ The vector ~~as claimed in any one of the preceding~~of ~~claims~~ claim 1 wherein the vector further comprises nucleic acid encoding a second selectable marker.

29. (currently amended) ~~A~~ The vector ~~as claimed in any one of the preceding~~ ~~claims~~of claim 1 wherein the vector is a DNA vector, particularly a dsDNA vector.

30. (original) Plasmid RL1.dIRES-GFP (ECACC accession number 03090303).

31. (currently amended) ~~A~~ The vector ~~as claimed in any one~~ of ~~the preceding claims~~ claim 1 wherein the vector is an expression vector.

32. (currently amended) A method of generating a herpes simplex virus which expresses a nucleotide sequence of interest, or polypeptide thereby encoded, comprising the step of culturing a selected herpes simplex virus with ~~a~~ the vector ~~as claimed in any one~~ of ~~claims~~claim 1 ~~to 31~~, thereby integrating components (a), (b) and (c) of said vector at said predetermined insertion site in the genome of the selected herpes simplex virus.

33. (original) The method of claim 32 wherein said herpes simplex virus is an HSV-1 or HSV-2.

34. (currently amended) The method of claim 32 ~~or 33~~ wherein the integrated components disrupt a protein coding sequence resulting in inactivation or lack of expression of the respective gene product in the generated virus.

35. (currently amended) The method ~~of any one~~ of ~~claims~~claim 32 ~~to 34~~ wherein the generated herpes simplex virus:

_____is a gene specific null mutant;

_____is an ICP34.5 null mutant;

_____lacks only one expressible ICP34.5 gene;

_____is non-neurovirulent; or

_____a combination of two or more thereof.

36. – 39. (cancelled)

40. (currently amended) A medicament comprising the vector ~~as claimed in any one~~ of claim 1 ~~claims 1 to 31 for use in a method of medical treatment~~.

41. – 45. (cancelled)

46. (currently amended) ~~The use claimed in claim 45 wherein said~~A medicament ~~comprises~~ comprising a mutant herpes simplex virus generated using ~~said~~ the vector of claim 1.

47. (currently amended) A kit of parts comprising a first container having a quantity of ~~a~~ the vector ~~as claimed in any one of claims~~claim 1 ~~to 31~~ and a second container comprising a quantity of herpes simplex virus genomic DNA.

48. (currently amended) An herpes simplex virus (HSV) wherein the herpes simplex virus comprises a nucleic acid cassette integrated in the RL1 locus of the HSV genome comprising nucleic acid encoding:

(I.)

(a) one or a plurality of insertion sites; and

(b) a ribosome binding site or a regulatory nucleotide sequence, and a

(c) marker,

wherein the nucleic acid encoding the one or plurality of insertion sites is/are arranged upstream (5′) of the ribosome binding site or the regulatory nucleotide sequence and the nucleic acid encoding the ribosome binding site or the regulatory nucleotide sequence is arranged upstream (5′) of the marker; or

(II.)

(a) a nucleotide sequence of interest; and nucleic acid encoding:

(b) a ribosome binding site or a regulatory nucleotide sequence; and

(c) a marker,

wherein the nucleotide sequence of interest is arranged upstream (5′) of the ribosome binding site or the regulatory nucleotide sequence and the ribosome binding site or the regulatory nucleotide sequence is arranged upstream (5′) of the marker.

49. (cancelled)

50. (currently amended) ~~A~~ The ~~vector~~ herpes simplex virus ~~as claimed in~~of claim 48 ~~or claim 49~~ wherein the ribosome binding site comprises an internal ribosome entry site (IRES).

51. (currently amended) ~~An~~ The herpes simplex virus ~~as claimed in any one~~ of ~~claims~~claim 48~~49 or 50~~ wherein a transcription product of the cassette is a bi- or poly- cistronic transcript comprising a first cistron encoded by the nucleotide sequence of interest and a second cistron encoded by the marker nucleic acid wherein the ribosome binding site is located between said first and second cistrons.

52. – 53. (cancelled)

54. (currently amended) ~~An~~ The herpes simplex virus ~~as claimed in~~of claim 48~~52 or claim 53~~ wherein said regulatory nucleotide sequence is operably linked to said marker.

55. (currently amended) ~~An~~ The herpes simplex virus ~~as claimed in any one~~ of ~~claims~~claim 48~~52 to 54~~ wherein said regulatory nucleotide sequence comprises a constitutive or inducible promoter.

56. (currently amended) ~~An~~ The herpes simplex virus ~~as claimed in any one~~ of ~~claims~~ 49claim 48 ~~to 51 or 53 to 55~~ wherein the nucleotide sequence of interest encodes an heterologous polypeptide.

57. (currently amended) ~~An~~ The herpes simplex virus as claimed in claim 56 wherein the heterologous polypeptide is selected from the group consisting of: a bacterial polypeptide; a mammalian polypeptide; a human polypeptide.

58. (currently amended) ~~An~~ The herpes simplex virus as claimed in claim 56 wherein the heterologous polypeptide is selected from the group consisting of: Sodium iodide symporter (NIS); Nitroreductase (NTR); *E. coli* NTR; Endothelial nitric oxide synthase (eNOS); Granulocyte Macrophage Colony-Stimulating Factor (GM-CSF); a cytokine.

59. (currently amended) ~~An~~ The herpes simplex virus ~~as claimed in any one~~ of ~~claims~~claim 48~~49 to 51 or 53 to 55~~ wherein the nucleotide sequence of interest encodes a selected antisense nucleic acid or siRNA.

60. (currently amended) ~~An~~ The herpes simplex virus ~~as claimed in any one of claims~~ claim 48~~49, 50, 51, 53, 56 to 59~~ wherein the cassette further comprises a regulatory nucleotide sequence located upstream (5′) of the nucleotide sequence of interest which has a role in regulating transcription of the nucleotide sequence of interest.

61. (currently amended) ~~An~~ The herpes simplex virus ~~as claimed in any one~~ of ~~claims~~claim 48~~, 50, 51, 52, 54 or 55~~ wherein the cassette further comprises a regulatory nucleotide sequence located upstream (5′) of the insertion site(s).

62. (currently amended) ~~An~~ The herpes simplex virus ~~as claimed in any one~~ of ~~claims~~claim 48~~, 50, 51, 52, 54, 55 or 61~~ wherein the cassette comprises a plurality of said insertion sites.

63. (currently amended) ~~An~~ The herpes simplex virus ~~as claimed in any one~~ of ~~claims~~claim 48~~, 50, 51, 52, 54, 55, 61 or 62~~ wherein each insertion site is formed by nucleic acid encoding a restriction endonuclease site.

64. (currently amended) ~~An~~ The herpes simplex virus ~~as claimed in any one~~ of ~~claims~~claim 48~~, 50, 51, 52, 54, 55, 61, 62 or 63~~ wherein the insertion sites comprise one or more of the ClaI, BglII, NruI and XhoI restriction endonuclease sites.

65. (currently amended) ~~An~~ The herpes simplex virus ~~as claimed in any one~~ of ~~claims~~claim 48~~ to 64~~ wherein the nucleic acid cassette is integrated in the RL terminal or internal repeat region of the genome of the selected HSV.

66. (currently amended) ~~An~~ The herpes simplex virus ~~as claimed in any one~~ of ~~claims~~claim 48 ~~to 65~~ wherein the nucleic acid cassette is integrated at a site formed in, or comprising all or a part of, the ICP34.5 protein coding sequence of the genome of a selected herpes simplex virus.

67. (currently amended) ~~An~~ The herpes simplex virus ~~as claimed in any one~~ of ~~claims~~claim 48 ~~to 66~~ wherein the nucleic acid cassette is integrated in the genomic nucleotide sequence of the ICP34.5 gene of a herpes simplex virus.

68. (currently amended) ~~An~~ The herpes simplex virus ~~as claimed in any one~~ of ~~claims~~claim 48 ~~to 67~~ wherein the nucleic acid cassette is integrated in the genomic nucleotide sequence encoding the ICP34.5 gene product of a herpes simplex virus.

69. (currently amended) ~~An~~ The herpes simplex virus ~~as claimed in any one~~ of ~~claims~~claim 48 ~~to 68~~ wherein the marker is a defined nucleotide sequence encoding a polypeptide.

70. (currently amended) ~~An~~ The herpes simplex virus ~~as claimed in any one~~ of ~~claims~~claim 48 ~~to 69~~ wherein the marker comprises the Green Fluorescent Protein (GFP) protein coding sequence or the enhanced Green Fluorescent Protein (EGFP) protein coding sequence.

71. (currently amended) ~~An~~ The herpes simplex virus ~~as claimed in any one~~ of ~~claims~~claim 48 ~~to 68~~ wherein the marker comprises a defined nucleotide sequence detectable by hybridisation under high stringency conditions with a corresponding labelled nucleic acid probe.

72. (currently amended) ~~An~~ The herpes simplex virus ~~as claimed in any one~~ of ~~claims~~claim 48 ~~to 71~~ wherein the cassette further comprises nucleic acid encoding a polyadenylation sequence located downstream (3′) of the nucleic acid encoding the marker.

73. (currently amended) ~~An~~ The herpes simplex virus as claimed in claim 72 wherein the polyadenylation sequence comprises the Simian Virus 40 (SV40) polyadenylation sequence.

74. (currently amended) ~~An~~ The herpes simplex virus ~~as claimed in any one~~ of ~~claims~~claim 48 ~~to 73~~ wherein the cassette disrupts a protein coding sequence in the HSV genome resulting in inactivation of the respective gene product.

75. (currently amended) ~~An~~ The herpes simplex virus ~~as claimed in any one~~ of ~~claims~~claim 48 ~~to 74~~ wherein the herpes simplex virus is a mutant of HSV-1 or HSV-2.

76. (currently amended) ~~An~~ The herpes simplex virus ~~as claimed in any one~~ of ~~claims~~claim 48 ~~to 75~~ wherein the herpes simplex virus is a mutant of one of HSV-1 strains 17 or F or HSV-2 strain HG52.

77. (currently amended) ~~An~~ The herpes simplex virus ~~as claimed in any one~~ of ~~claims~~claim 48 ~~to 76~~ which is a gene specific null mutant.

78. (currently amended) ~~An~~ The herpes simplex virus ~~as claimed in any one~~ of ~~claims~~claim 48 ~~to 77~~ which is an ICP34.5 null mutant.

79. (currently amended) ~~An~~ The herpes simplex virus ~~as claimed in any one~~ of ~~claims~~claim 48 ~~to 78~~ which lacks at least one expressible ICP34.5 gene.

80. (currently amended) ~~An~~ The herpes simplex virus ~~as claimed in any one~~ of ~~claims~~claim 48 ~~to 76~~ which lacks only one expressible ICP34.5 gene.

81. (currently amended) ~~An~~ The herpes simplex virus ~~as claimed in any one~~ of ~~claims~~claim 48 ~~to 79~~ which is non-neurovirulent

82. (currently amended) ~~An~~ The herpes simplex virus ~~as claimed in any one~~ of ~~claims~~claim 48 ~~to 81~~ for use in a method of medical treatment.

83. (currently amended) ~~An~~ The herpes simplex virus ~~as claimed in any one~~ of ~~claims~~claim 48 ~~to 81~~ for use in the treatment of cancer.

84. (currently amended) ~~An~~ The herpes simplex virus ~~as claimed in any one of claims~~claim 48 ~~to 81~~ for use in the oncolytic treatment of a tumour.

85. (cancelled)

86. (currently amended) A method of lysing or killing tumour cells *in vitro* or *in vivo* comprising the step of administering to a patient in need of treatment a therapeutically effective amount of ~~an~~ the herpes simplex virus ~~as claimed in any one of claims~~claim 48 ~~to 81~~.

87. (currently amended) A medicament, pharmaceutical composition or vaccine comprising ~~an~~ the herpes simplex virus ~~as claimed in any one of claims~~claim 48 ~~to 81~~.

88. (currently amended) ~~A~~ The medicament, pharmaceutical composition or vaccine as claimed in claim 87 further comprising a pharmaceutically acceptable carrier, adjuvant or diluent.

89. (original) A method of generating a nucleic acid vector comprising the steps of:

i) providing a first nucleotide sequence comprising a predetermined second nucleotide sequence corresponding to a selected nucleotide sequence in the RL1 locus of the genome of a selected Herpes simplex virus; and

ii) inserting nucleotide sequence(s) in said second nucleotide sequence encoding:

a) one or a plurality of insertion sites and/or a nucleotide sequence of interest; and

b) a ribosome binding site or a regulatory nucleotide sequence; and

c) a marker,

wherein the insertion site(s)/nucleotide sequence of interest is arranged upstream (5′) of the ribosome binding site/ regulatory nucleotide sequence and the ribosome binding site / regulatory nucleotide sequence is arranged upstream (5′) of the marker.

90. (original) The method of claim 89 wherein the inserted nucleotide sequence(s) separates the second nucleotide sequence into two vector flanking sequences, the inserted nucleotide sequences forming a cassette therebetween.

91. (currently amended) The method as claimed in claim 89 ~~or claim 90~~ wherein the second nucleotide sequence corresponds to a nucleotide sequence in the RL terminal or internal repeat region of the genome of the selected herpes simplex virus.

92. (currently amended) The method ~~as claimed in any one~~ of ~~claims~~claim 89 ~~to 91~~ wherein the second nucleotide sequence corresponds to all or a part of the ICP34.5 protein coding sequence of the genome of the selected herpes simplex virus.

93. (currently amended) The method ~~as claimed in any one~~ of ~~claims~~claim 89 ~~to 92~~ wherein said second nucleotide sequence comprises a contiguous portion of nucleotide sequence of the ICP34.5 gene of the selected herpes simplex virus.

94. (currently amended) The method ~~as claimed in any one~~ of ~~claims~~claim 91 ~~to 93~~ wherein said second nucleotide sequence comprises a contiguous portion of nucleotide sequence encoding the ICP34.5 gene product of the selected herpes simplex virus.

95. (currently amended) The method ~~as claimed in any one~~ of claim 89 ~~to 94~~ wherein the second nucleotide sequence has at least 60% sequence identity to the corresponding sequence in the viral genome.

96. (currently amended) The method ~~as claimed in any one~~ of ~~claims~~claim 89 ~~to 94~~ wherein said second nucleotide sequence hybridises to the corresponding nucleotide sequence in the viral genome, or its complement, under high or very high stringency conditions

97. (original) A method of generating a mutant herpes simplex virus (HSV) comprising inserting a nucleic acid cassette comprising nucleotide sequence(s) encoding:

a) one or a plurality of insertion sites and/or a nucleotide sequence of interest; and

b) a ribosome binding site or a regulatory nucleotide sequence; and

c) a marker

into a predetermined insertion site in the RL1 locus of the genome of a selected HSV, wherein the insertion site(s)/nucleotide sequence of interest is arranged upstream (5′) of the ribosome binding site/ regulatory nucleotide sequence and the ribosome binding site/ regulatory nucleotide sequence is arranged upstream (5′) of the marker.

98. (currently amended) The method of claim 97 wherein said method comprises the steps of:

i) providing ~~a~~ the vector ~~as claimed in any one~~ of ~~claims~~claim 1 ~~to 31~~;

ii) where the vector is a plasmid, linearising the vector; and

iii) co-transfecting a cell culture with the linearised vector and genomic DNA from said selected HSV.

99. (original) The method of claim 98 wherein said co-transfection is carried out under conditions effective for homologous recombination of said cassette into an insertion site in the viral genome.

100. (currently amended) The method of ~~any one of claims~~claim 97 ~~to 99~~ wherein said method further comprises one or more of the steps of:

1) screening said co-transfected cell culture to detect mutant HSV expressing said marker; and/or
2) isolating said mutant HSV; and/or
3) screening said mutant HSV for expression of the nucleotide sequence of interest or the RNA or polypeptide thereby encoded; and/or
4) screening said mutant HSV for lack of an active gene product; and/or
5) testing the oncolytic ability of said mutant HSV to kill tumour cells in vitro.

101. (currently amended) ~~A~~ The method ~~as claimed in any one~~ of ~~claims~~claim 97 ~~to 100~~ wherein the nucleotide sequence of interest is heterologous to the selected herpes simplex virus.

102. (currently amended) The method ~~as claimed in any one~~ of ~~claims~~claim 97 ~~to 100~~ wherein the nucleotide sequence of interest encodes an heterologous polypeptide.

103. (original) The method as claimed in claim 102 wherein the heterologous polypeptide is selected from the group consisting of: a bacterial polypeptide; a mammalian polypeptide; a human polypeptide.

104. (currently amended) The method as claimed in claim 102 wherein the heterologous polypeptide is selected from the group consisting of: Sodium iodide symporter (NIS); Nitroreductase (NTR); *E. coli* NTR; Endothelial nitric oxide synthase (eNOS); Granulocyte Macrophage Colony-Stimulating Factor (GM-CSF); a cytokine.

105. (original) The method as claimed in claim 101 wherein the nucleotide sequence of interest encodes a selected antisense nucleic acid or siRNA.

106. (currently amended) An herpes simplex virus generated by the method of ~~any one of claims~~claim 97 ~~to 105~~.

107. (currently amended) An herpes simplex virus gene specific null mutant generated by the method of ~~any one of claims~~claim 97 ~~to 105~~.

108. (currently amended) An herpes simplex virus ICP34.5 null mutant generated by the method of ~~any one of claims~~claim 97 ~~to 105~~.